

HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

TEST PLAN

For the

MONONITROANILINE CATEGORY

CAS Number 88-74-4; Benzeneamine, 2-nitro-

CAS Number 100-01-6; Benzeneamine, 4-nitro-

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EXECUTIVE SUMMARY

Solutia Inc. voluntarily submits the following Category Justification, Screening Information Data (Robust Summaries) and Test Plan for review under the Environmental Protection Agency's High Production Volume (HPV) Chemicals Challenge Program. The category, entitled "Mononitroanilines" consists of two members, Benzeneamine, 2-nitro, also known as 2-Nitroaniline (CAS No. 88-74-4) and Benzeneamine, 4-nitro, also known as 4-Nitroaniline (CAS No. 100-01-6). This category is justified on the basis of chemical structure similarity, as well as similarity of basic screening data, as provided in an initial assessment of physico-chemical properties, environmental fate and human and environmental effects.

A substantial amount of data exists to evaluate the potential hazards associated with this Category of chemicals. Use of key studies available from data already developed or derived from recommended estimation models provide adequate support to characterize each Endpoint in the HPV Chemicals Challenge Program without the need for additional testing.

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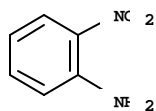
TEST PLAN FOR MONONITROANILINES

I. INTRODUCTION AND IDENTIFICATION OF CATEGORY MEMBERS

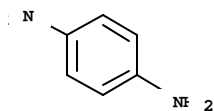
Under EPA's High Production Volume (HPV) Chemicals Challenge Program, Solutia Inc. has committed to voluntarily compile basic screening data on two chemicals of similar structure, namely Benzeneamine, 2-nitro (known as 2-nitroaniline or ONA) and Benzeneamine, 4-nitro (known as 4-nitroaniline or PNA). Solutia Inc. believes that a category of Mononitroanilines is scientifically justifiable. The data included in this category involve physicochemical properties, environmental fate, and human and environmental effects of the chemicals in this Category, as defined by the Organization for Economic Cooperation and Development (OECD). Most of the information provided comes from existing data developed on behalf of Solutia Inc., much of which has already been submitted to the US EPA under auspices of sections of the Toxic Substances Control Act and is available through TSCATS; additional information can be found in the published scientific literature or from recommended estimation models. This submission fulfills Solutia's obligation to the HPV Challenge Program for these two chemicals.

A. Structure and Nomenclature

The members of this family of Mononitroanilines, include the following chemicals:



- a. Benzeneamine, 2-nitro-
CAS No. 88174-4
Synonyms: 2-Nitroaniline; 1-Amino-2-nitrobenzene; ortho-nitroaniline; o-nitroaniline; ONA;



- b. Benzeneamine, 4-nitro-
CAS No. 100-01-6
Synonyms: 4-nitroaniline; 1-Amino-4-nitrobenzene; para-nitroaniline; p-nitroaniline; PNA;

B. Manufacturing & Use

Both p-Nitroaniline (PNA) and o-Nitroaniline (ONA) are manufactured by a single US producer, Solutia Inc., at a single manufacturing site in an essentially closed, continuous process. Only a few employees are involved in the manufacturing operations and have minimal potential for skin or airborne exposure, which occur chiefly during material transfer operations.

Both PNA and ONA produce methemoglobinemia in human and animals (Linch, 1974; Watanabe et al, 1976) and are known to be hazardous after dermal contact. Addition of the nitro group in the *para* position to the aniline molecule results in the formation of the more toxic compound. To minimize the potential for adverse health effects due to methemoglobinemia resulting from occupational exposure via inhalation or skin absorption, a TLV ® of 3 mg/m³ has been established for PNA (ACGIH, 2001). While comparative toxicity and occupational experience indicate that ONA produces less toxicity and a lower risk of methemoglobinemia, an internal Solutia Inc. occupational standard of 3 mg/m³ has also been set for this chemical. In both cases, specific manufacturing procedures and practices have been established to minimize occupational exposure potential.

Both Mononitroanilines, para-Nitroaniline (PNA) and ortho-Nitroaniline (ONA), are important chemical intermediates which serve as basic building blocks for the ultimate manufacture of numerous industrial chemicals. For example, PNA is utilized in preparation of antioxidants, antiozonants, and dyes and pigments while ONA is converted to polymer additives, veterinary pharmaceuticals and water-treatment chemicals.

PNA and ONA are sold to a limited number of customers at a few US processing sites for the express purpose of full chemical conversion into other industrial chemicals. There are no known or suspected consumer exposures to either PNA or ONA resulting from TSCA-related activities, as they are fully consumed as chemical intermediates. Loss to the atmosphere or from non-POTW aqueous streams during manufacturing or processing is minimal. Hence, very limited occupational or environmental exposure is expected to occur.

II. CATEGORY JUSTIFICATION

For purposes of the HPV Challenge Program, EPA has provided guidance as to the definition and justifications to be used in selection of a chemical Category (US EPA, 1999c). The definition states that a chemical category should be “a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity”. Solutia Inc. has opted to form the Mononitroaniline Category with this guidance in mind.

Common Structure

The two chemicals selected for inclusion in this category are isomeric forms of the same base chemical, nitroaniline. Hence, they are of common structure.

Common Functional Groups

Each of these amino compounds are aromatic hydrocarbons for which one benzene ring hydrogen has been replaced by a nitro (NO₂) radical and one benzene ring hydrogen further replaced with an amino (NH₂) group; the position (either *ortho* to or *para* to the nitro grouping) of the ring placement of the amino grouping is the only structural difference between these two chemicals. For the most part, these compounds are similar in chemical properties, as well as in their pharmacological or toxicological effects. As such these effects are modified to a greater or lesser degree by the location of the substituent radical (Beard and Noe, 1982).

Similar or even Identical Properties or Hazards

Physicochemical properties of these two isomeric forms of the same chemical are quite similar. The physical form of both is crystalline and their molecular weights and specific gravity are identical. Other parameters are similar, but not identical.

A summary of available physicochemical data can be found in Table 3.

Environmental Fate data are summarized in Table 4. As shown, a large body of published information exists in this data category. Whether measured or estimated, there appears close agreement in each of the HPV Endpoints recorded for both chemicals in this category.

Comparative aquatic toxicity of both members of this Category can be found in Table 5. As shown, a similar degree of toxicity has been observed across the multiple test species included in this dataset.

Tables 6 - 9 summarize the comparative mammalian toxicity of both of these chemicals. It is well recognized that both chemicals possess a similar mode of action. Their toxicity is characterized by a common and outstanding property, i.e., the ability to form methemoglobin (Beard and Noe, 1982) in both humans and animals. However, there are marked species differences in susceptibility to methemoglobinemia with humans being decidedly more affected than rodent species. Thus, results of acute toxicity studies in rodents are not considered fully representative of the high acute toxicity to humans which can be elicited by these chemicals. On the basis of past human experience, where dermal contact or inhalation exposures resulted in incidences of methemoglobinemia, unusually diligent care has been taken to insure proper handling of both chemicals (each treated equally) during manufacture, shipment, disposal and use.

Thus, similarities in the biological mode of action and the extensive comparative data sets presented support use of a Category approach for these chemicals.

III. TEST PLAN RATIONALE

The information obtained and included to support this Test Plan have come from either 1) internal studies conducted by/or for Solutia Inc. (or its predecessor Monsanto Co.), 2) have been extracted from the scientific literature either as primary references or as found in well-accepted, peer-reviewed reference books, or 3) were estimated using environmental models accepted by the US EPA (1999b) for such purposes. This initial assessment includes information on physicochemical properties, environmental fate, and human and environmental effects associated with both members of this Category. The data used to support this program include those endpoints identified by the US EPA (1998); key studies have been identified for each data Endpoint and summarized in Robust Summary form and included in Section VII of this dossier.

All studies were reviewed and assessed for reliability according to standards specified by Klimisch *et al* (1997), as recommended by the US EPA (1999a). The following criteria were used for codification:

1. Reliable without Restriction - Includes studies which comply with US EPA and/or OECD-accepted testing guidelines, which were conducted using Good Laboratory Practices (GLPs) and for which test parameters are complete and well documented,

2. **Reliable with Restriction** – Includes studies which were conducted according to national/international testing guidance and are well documented. May include studies conducted prior to establishment of testing standards or GLPs but meet the test parameters and data documentation of subsequent guidance; also includes studies with test parameters which are well documented and scientifically valid but vary slightly from current testing guidance. Also included were physical-chemical property data obtained from reference handbooks as well as environmental endpoint values obtained from an accepted method of estimation (i.e. EPIWIN).
3. **Not Reliable** – Includes studies in which there are interferences in either the study design or results that provide scientific uncertainty or where documentation is insufficient.
4. **Not Assignable** – This designation not used in this dossier.

Those studies receiving a Klimisch rating of 1 or 2 are considered adequate to support data assessment needs in this Dossier. Those key studies selected for inclusion are considered typical of the Endpoint responses observed in other studies of a similar nature and design, which were identified during our search of the literature; additional references can be found in the current ECB IUCLID dossiers for p-Nitroaniline (2000) and o-Nitroaniline (2000), as referenced below.

IV. TEST PLAN SUMMARIES AND CONCLUSIONS

The referenced available data for each Category member has been placed in an Endpoint-specific matrix and summarized individually in Table 1 (PNA) and Table 2 (ONA). Substantial data exists for each chemical to evaluate its potential hazards in this screening level assessment. Where an HPV Endpoint has been untested, the need for testing has been assessed (1) with the understanding that these chemicals behave in a similar and/or predictable manner, and (2) by interpolation (i.e. Read-Across technique) between data from other key studies already available. Thus, we have used preexisting data, where possible, to support our assessment of potential hazards of the chemicals in this category and avoid the unnecessary testing of additional laboratory animals.

Conclusion: All HPV Endpoints have been satisfied for both PNA and ONA with data from studies that were either well documented, used OECD guideline methods and conducted in accord with GLPs, or were estimated from acceptable estimation modeling programs. Known properties or use of Read Across” were

used sparingly to support a limited number of endpoints. Hence, no further testing for any of the HPV endpoints is deemed necessary (Tables 1 and 2).

Physical-chemical property values (Melting Point, Boiling Point, Vapor Pressure, Partition Coefficient and Water Solubility) for both PNA and ONA were obtained from reputable references and cited as an Accepted or Peer Reviewed value in the Hazardous Substances Data Bank – p-Nitroaniline (2002) or the Hazardous Substances Data Bank – o-Nitroaniline (2002). They were given a classification of “2-Reliable with restrictions”.

Environmental Fate values describing Photodegradation (PNA only) and Transport (Fugacity) for both PNA and ONA were obtained using a computer estimation – modeling program (EPIWIN, 2002) recommended by EPA and classified as “2-Reliable with restrictions”; Photodegradation study data was used for ONA and Biodegradation data for PNA and ONA were characterized in a well documented study conducted according to ASTM/EPA guidelines, which since have been codified and are similar to OECD test #301 guidance and thus also classified as “2-Reliable with restrictions”. No Stability in Water (hydrolysis) data was found for either ONA or PNA, nor could values be calculated using EPIWIN, as these chemicals are known to be resistant to hydrolysis.

Ecotoxicity Endpoints for PNA and ONA have been fulfilled with studies that were conducted either according to OECD test guidelines or followed US EPA test guidance consistent with OECD test guidelines. All studies were well documented and were designated “1-Reliable without restriction”.

Mammalian Toxicity Endpoints, including Acute Toxicity, Repeated Dose Toxicity, Ames Mutagenicity and Chromosomal Aberration Testing, for both PNA and ONA have been fulfilled by way of tests that either conformed directly to OECD test guidance or followed test designs similar to OECD guidance.

The Acute Toxicity Endpoint for ONA is supported by an acute inhalation study that followed OECD guideline 403 and was considered “1-Reliable without restriction”. PNA is supported by an acute oral toxicity study of sound scientific merit and designated “2-Reliable with restrictions”, as small differences existed in methodology vs. OECD # 401.

A 90-Day oral rat toxicity study meeting OECD test guideline # 408, and deemed “1-Reliable without restriction” supports the Repeated Dose Endpoint for PNA. Tandem (initial and subsequent follow-on study) 4-week inhalation studies conducted with ONA jointly meet OECD test guideline 412 and thus fulfill this data Endpoint;

Ames mutagenicity tests with PNA and ONA followed study designs equivalent to OECD guideline # 471 and have been designated “1-Reliable without restriction” and “2-Reliable with restrictions”, respectively. Mouse Micronucleus Assays, conducted with PNA and ONA, respectively, followed OECD test guideline # 474 and were each designated “1-Reliable without restriction”.

A 2-Generation Reproduction Study fulfills the HPV requirements for the last mammalian toxicity Endpoint for PNA. This study meets OECD test guideline # 416 and has been classified as “1-Reliable without restriction”. No similar Reproductive toxicity testing has been identified with ONA, although a fully acceptable (“1-Reliable without restriction”) rat developmental toxicity study with ONA has been conducted. Use of the “Read-across” concept (i.e. determination of the need to fulfill this data requirement based on substitutive use of available data from a similar, closely related chemical...in this case PNA) obviates the need for additional testing for ONA. While Repeated Dose Toxicity testing with ONA appears insufficient in duration (only 4 weeks rather than 13 weeks) to meet EPA/OECD guidance for completion of the Reproductive Toxicity Endpoint (US EPA, 1998), it is noteworthy that there is an absence of testicular effects seen (1) with ONA in multiple studies of less than 90 days duration (by two exposure routes) and (2) in numerous studies of greater than 90 days duration (including chronic testing) with PNA.

Based on the conclusions as outlined above on HPV Endpoint assessment, following is a tabular depiction of data availability and testing recommendations for p-Nitroaniline (PNA) (Table 1) and o-Nitroaniline (ONA) (Table 2).

Table 1. Test Plan Matrix for para-Nitroaniline (PNA)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept- Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	-	Y	N
Boiling Point	Y	N	N	R	-	Y	N
Vapor Pressure	Y	N	N	R	-	Y	N
Partition Coefficient	Y	N	N	R	-	Y	N
Water Solubility	Y	N	N	R	-	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	-	Y	Y	N
Stability in Water	N	N	N	-	N	-	N
Biodegradation	Y	N	N	Y	-	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	-	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	Y	Y	-	-	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	Y	Y	-	-	Y	N
Acute Toxicity to Aquatic Plants	Y	Y	L	-	-	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	N	N	Y	-	Y	N
Repeated Dose Toxicity	Y	Y	Y	-	-	Y	N
Genetic Toxicity – Mutation (Ames)	Y	Y	Y	-	-	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	Y	Y	-	-	Y	N
Reproductive Toxicity	Y	Y	Y	-	-	Y	N
Developmental Toxicity	Y	Y	Y	-	-	Y	N

Y = Yes; N = No; L = Likely, but not specified; R = Reputable Reference;
 ND = No information available; - = Not applicable

Table 2. Test Plan Matrix for ortho-Nitroaniline (ONA)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept- Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	-	Y	N
Boiling Point	Y	N	N	R	-	Y	N
Vapor Pressure	Y	N	N	R	-	Y	N
Partition Coefficient	Y	N	N	R	-	Y	N
Water Solubility	Y	N	N	R	-	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	-	Y	N
Stability in Water	N	N	N	-	N	-	N
Biodegradation	Y	N	N	Y	-	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	-	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	Y	L	-	-	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	Y	Y	-	-	Y	N
Acute Toxicity to Aquatic Plants	Y	Y	L	-	-	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	Y	Y	-	-	Y	N
Repeated Dose Toxicity	Y	Y	Y	-	-	Y	N
Genetic Toxicity – Mutation (Ames)	Y	N	N	Y	-	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	Y	Y	-	-	Y	N
Reproductive Toxicity	N	-	-	-	-	C	N
Developmental Toxicity	Y	Y	Y	-	-	Y	N

Y = Yes; N = No; L = Likely, but not specified; R = Reputable Reference;
C = Read-across from available data on PNA; - = Not applicable

V. Data Set Summaries and Evaluations

The key studies used in this assessment to fulfill the HPV requirements for both PNA and ONA have been placed in an Endpoint-specific matrix, and further discussed below. As a number of studies supporting many of these Endpoints exist for each Mononitroaniline, key studies were selected based on their representative presentation of data characterization as well as their reliability. Robust Summaries for each study referenced can be found in Section VII of this dossier.

A. Chemical/Physical Properties

A large number of studies are available summarizing the **Physical-Chemical** properties associated with both of these Mononitroanilines. They can be found in ECB IUCLID Dossiers for p-Nitroaniline (2000) and o-Nitroaniline (2000). Table 3 contains those values that are considered to best depict the consensus of results found in most key sources used to define the characteristics of each of these Mononitroanilines. They have been obtained from reputable reference books and cited in peer-reviewed data sources; thus, they are considered “2-Reliable with restrictions”. A Robust Summary has been prepared for each of the references included in Table 3.

In summary, PNA and ONA are solid entities at room temperature and possess low vapor pressures. They have a moderate partition coefficient and are moderately soluble in water.

Conclusion: Sufficient data exists to fully characterize the Physical-Chemical properties of each of these Mononitroanilines. All HPV data requirements for this Endpoint have been met and no further data collection is planned.

Table 3. Selected Physical Properties of Mononitroanilines

Chemical	Boiling Pt. (°C.)	Melting Pt. (°C.)	Vapor Pressure (hPa @ 25 °C)	Water Solubility (mg/L)	Partition Coefficient (Log Kow)
o-Nitroaniline CAS No. 88-74-4	284	71.5	0.0368	1470 @ 25 °C.	1.85
p-Nitroaniline CAS No. 100-01-6	332	146	0.0053	724 @ 25 °C.	1.39

B. Environmental Fate and Biodegradation

A well-conducted Semi-Continuous Activated Sludge (SCAS) Biodegradability study has been conducted to compare the biodegradation potential of PNA and ONA; it has been summarized in the Robust Summary section of this Dossier and cited in Table 4 below.

While conducted prior to inception of standardized international guidelines for **Biodegradability** testing and GLPs, this study followed similar standards for conduct subsequently codified into OECD guideline 301 and GLP documentation. Thus, this study is considered to be “2-Reliable with restrictions”. We have incorporated the use of estimation models found in EPIWIN (2002) for determination of **Photodegradation** for PNA and Transport Between Environmental Compartments (**Fugacity**), using model Level III, and employing measured values, where possible, as recommended by the US EPA. Thus, they have been classified as “2-Reliable with restrictions”. The Photodegradation study with ONA was classified as “2-Reliable with restriction”. These estimates have also been included in Table 4 and are cited in the Robust Summary section of this Dossier; data entries into the Level III fugacity model have been included in the Robust Summaries for validation of output. No values have been identified for either ONA or PNA to define their **Stability in Water** (hydrolysis). Further no such values could be calculated using EPIWIN (2002) as both ONA and PNA have only aromatic nitro and aromatic amine functional groups, both of which are listed in Lyman et al. (1990) as Generally Resistant to Hydrolysis. Thus, “[t]esting for Stability in Water is not needed for substances generally recognized to have molecular structures or possess only functional groups that are generally known to be resistant to hydrolysis” (OECD, 2002).

Conclusion: Sufficient information exists to characterize the Environmental Fate and Biodegradation of each of these Mononitroanilines. Where experimental data do not exist, use of an estimation model (EPIWIN) recommended by EPA provided necessary information or the rationale lack of need for testing has already been recognized. Thus, all HPV data requirements for this Endpoint are met and no further data collection is planned.

Table 4. Comparison of Biodegradation Endpoints for Category Members

Chemical	Biodegradation Rate	Stability in Water	Photodegradation	Fugacity (%)
o-Nitroaniline CAS No. 88-74-4	7% Primary Degrad. (SCAS)	n.d.	T1/2 = 9.5 hr	Air- 0.5 Water- 36.1 Soil- 63.3 Sediment-0.1
p-Nitroaniline CAS No. 100-01-6	82% Primary Degrad. (SCAS)	n.d.	T1/2 = 9.5 hr	Air- 0.6 Water- 36.8 Soil- 62.6 Sediment-0.01

n.d. = no data available

To summarize the Environmental fate of these Mononitroanilines, PNA and ONA should readily degrade in the vapor phase in the ambient atmosphere via reaction with photochemically produced OH⁻ radicals and thus exhibit a short half-life (Meylan and Howard, 1993)(Table 4). Aromatic amines and nitroaromatics are generally resistant to aqueous environmental hydrolysis (Lyman et al, 1982); therefore, estimations to determine hydrolysis in water could not be determined from use of an EPIWIN program

as no hydrolysable groups were found on the molecule (Table 4). Even in activated sludge testing, ONA is considered resistant to biodegradation, while PNA is considered “readily biodegradable” (Table 4). Similar studies cited in the IUCLID dossiers (ECB IUCLID on ONA, 2000, and PNA, 2000), also indicate a similar pattern of biodegradation capacity. Regression-derived estimates and experimentally-derived values of studies summarized in their respective IUCLID dossiers (2000) indicate that the bioconcentration potential of both ONA and PNA are low. Therefore, aquatic hydrolysis, volatilization from the aqueous environment and bioconcentration are of little importance (Lyman et al, 1982).

C. Aquatic Toxicity

Several references to acute fish, invertebrate and algal toxicity can be found in the ECB IUCLID documents for PNA (2000) and ONA (2000). Data presented in Table 5, and summarized in the Robust Summary section VII, depict the level of toxicity generally observed for these Endpoints within the overall dataset. Each of the studies selected was conducted according to OECD test guidelines (# 201, 202, or 203) or according to US EPA test guidance (ASTM/EPA) consistent with international guidance. Thus, they are considered “1-Reliable without restriction” even though no specific mention was made of their conduct employing GLPs. As these studies were published in peer-reviewed journals and were specifically identified as having been conducted in accord with OECD test methods, it is reasonable to assume that GLP guidance was also followed.

Conclusion: Sufficient data exists to fully characterize the Acute Aquatic Toxicity properties of each of these Mononitroanilines. All HPV data requirements for this Endpoint have been met and no further data collection is planned for either material.

Based on the values presented in Table 5, both PNA and ONA have a similar degree of acute toxicity to all three aquatic species; studies with *D. magna* proved to produce the lowest levels of toxicity, comparatively. Overall, PNA and ONA are considered to possess a low order of ecotoxicity.

Table 5. Comparison of Aquatic toxicity parameters for category members

Chemical	Fish LC 50 (mg/L)	Invertebrate LC50 (mg/L)	Algae EC50 (mg/L)
o-Nitroaniline CAS No. 88-74-4	19.5 (96-hr) (Zebrafish)	14.5 (48-hr) (Daphnia magna)	64.5 (48- hr)
p-Nitroaniline CAS No, 100-01-6	45 (96-hr) (R. trout)	20.0 (48-hr) (Daphnia magna)	54.9 (48-hr)

D. Mammalian Toxicity

1.0 Acute Toxicity

Key acute toxicity studies by multiple exposure routes were chosen from a number of other acute reports to represent the highest (most toxic) acute toxicity values identified from reliable sources. This was done specifically since acute toxicity studies with some laboratory animals are not considered sufficiently predictive of the acute hazards of these nitroanilines to humans, due to the resistance observed in lab animals to development of methemoglobinemia. All studies included in Table 6 were conducted specifically or in general agreement with OECD acute toxicity testing guidance and are considered either “1-Reliable without restriction” or “2-Reliable with restrictions”. While individual studies were identified as key studies (inhalation study for ONA and oral study for PNA) to fulfill this HPV Endpoint for each of the Category members, reliable studies involving other exposure routes are included as Supplemental information to provide as complete a summary as possible for this assessment. Other acute toxicity reports are also cited in the ECB IUCLID dossiers for both PNA (2000) and ONA (2000).

Table 6. Acute Mammalian Toxicity for Category members

Chemical	Oral LD50 (mg/kg)	Dermal LD50 (mg/kg)	Inhalation LC50 (mg/L)
o-Nitroaniline CAS NO. 88-74-4	2,050 (rat)	> 7,940 (rabbit)	> 2529 mg/m ³ (rat) - 4-hr. expos.
p-Nitroaniline CAS No. 100-01-6	1,400 (rat)	> 7,940 (rabbit)	-

Conclusion: Sufficient data from well-documented studies exist to meet the Acute Toxicity data set requirements for both members of this Category. Hence, no further acute toxicity testing is planned.

2.0 Repeated Dose Toxicity

PNA, the sentinel chemical in this Category, has been extensively evaluated in Repeated Dosing studies of various durations and by different exposure routes. Studies which fulfill the requirements for this HPV Endpoint are summarized in Table 7. Additional repeated dose studies, including a chronic oral rat study, a 13-week oral toxicity study in mice and a chronic toxicity/carcinogenicity study in mice are included in the ECB IUCLID – PNA (2000) dossier. The key study selected to fulfill this HPV Endpoint was the 90-day oral study in rats, which followed OECD Test Guideline 408 and is considered “1-Reliable without restriction”.

A consistent pattern of repeated dose PNA toxicity is apparent. Clinical observations, serum chemistry changes, organ weight differences and histopathological findings were all related to methemoglobin formation and compensatory processes that occurred as a

result. Further, these same toxicological effects were seen consistently (and to the exclusion of other effects) after 14 days on test, after 13 weeks of testing, or at interim or final sacrifice after lifetime exposure. Specifically, no treatment-related effects on male or female gonads (reproductive organs) were seen in any of the above studies; thus, these tissues are not considered as target organs for PNA.

Conclusion: The Repeated Dose HPV Endpoint for PNA is complete with conduct of a 13-week oral study in rats and no further testing is needed.

Table 7. Repeated Dose Toxicity Studies with Category Members

Chemical	Study Type	Dosages	Histopathology	Hematology/ Clinical Findings
o-Nitroaniline (ONA) CAS NO. 88-74-4	4-Week Rat inhal. 6 hr/d; 5d/wk 10/sex/group	93 mg/m ³ (males only) 73 mg/m ³ 28 mg/m ³ 10 mg/m ³	No treatment- Related findings	Serum methemoglobin Hematocrit Leukocytes Hemoglobin Erythrocytes Leukocytes Serum calcium Serum calcium NOEL
p-Nitroaniline (PNA) CAS No. 100-01-6	4-Week Rat inhal. 6 hr/d; 5d/wk 10/sex/group	90 mg/m ³ 30 mg/m ³ 10 mg/m ³	spleen wt. hemosiderosis & hematopoiesis in spleen & liver spleen wt. hemosiderosis & hematopoiesis in spleen spleen wt. hemosiderosis & hematopoiesis in spleen	methemoglobin anemia leukocytes methemoglobin anemia
p-Nitroaniline (PNA) CAS No. 100-01-6	90-Day Oral (gavage) 20/sex/group	30 mg/kg 10 mg/kg 3 mg/kg	hemosiderosis & hematopoiesis in spleen hemosiderosis & hematopoiesis in spleen hemosiderosis & hematopoiesis in spleen	methemoglobin anemia methemoglobin anemia methemoglobin anemia

ONA has been evaluated in a series of two 4-week repeated dose inhalation rat studies designed to provide comparative toxicological evaluation with a 4-week inhalation PNA study conducted concurrently and cited above (Table 7). Due to confounding use of a solvent in the first ONA study, i.e., ethylene glycol monoethyl ether (EGME, i.e. CELLOSOLVE), which was subsequently determined to produce effects on the testes, a follow-up study using a targeted design to assess this endpoint was performed with ONA (without EGME). The initial 4-week study was conducted according to GLPs and met OECD Test Guideline 412 study parameters. Due to the confounding use of EGME, this study is judged as “2-Reliable with restrictions”. Specifically, all study parameters measured (clinical signs, body weight, ophthalmology, blood chemistry, hematology, organ weights, microscopic pathology), EXCEPT for effects regarding the testes and the hematology findings are considered reliable. The rationale for this conclusion rests on the fact that all other study endpoints assessed were without effect even up to the highest level tested and hence, no effect of treatment was noted. The hematological effects noted at the high test level were consistent with a methemoglobin-forming chemical and thus were reevaluated (and confirmed as treatment-related) in the follow up study.

In order to assess the hematology and testicular findings seen in the first ONA inhalation study, a follow up study was conducted at two ONA doses, one was the low dose originally used and the other was a dose level in excess of that originally used. The follow-up study used only male rats and measured only hematological effects (noted in the earlier study) and testicular effects (weights and histopathology). This time the ONA atmosphere was generated without use of EGME. Results of this study affirmed the effects of ONA on hematology parameters seen in the original study at the high dose level, but also established that no effects on the testes occurred, either macroscopically or microscopically, when ONA alone was used. On this basis, the findings in this second study are considered “1-Reliable without restriction”. Thus, the gonadal effects seen in the original study were not reproduced when ONA was retested without use of EGME, even at a higher dose level than used in the first study, and confirmed that the original results were unrelated to ONA treatment. Subsequent to conduct of these studies the effects of EGME on the reproductive system appeared in the scientific literature (Barbee et al., 1984) providing further confirmation of this conclusion. A summary of the two, combined 4-week inhalation studies described above are included in Table 7, and summarized separately in the Robust Summary section of this Dossier.

To summarize, subchronic toxic effects with ONA were equivalent to those seen with PNA albeit to a lesser degree and were consistent with ONA’s diminished capacity to produce methemoglobinemia relative to PNA. There were no effects on male or female gonads seen with either Mononitroaniline. Komsta et al (1989) also reported no treatment-related effects associated with any of a comprehensive evaluation of biochemical, hematological and histopathological indices (including a lack of effect on gonads of either sex) following 14-day oral dosing of ONA to rats (ECB IUCLID – ONA, 2000).

Conclusion: Consideration of the two 4-Week inhalation studies in combination, the requirements for the Repeated Dose HPV Endpoint for ONA are complete and no further testing is needed.

3.0 Mutagenicity and Chromosomal Aberrations

Ames Test – p-Nitroaniline

PNA has been examined extensively in the Ames test. While a number of literature citations report the lack of mutagenic activity with PNA, the preponderance of evidence indicates that PNA expresses a weak mutagenic response in tester strain TA98 and the nitroreductase modified TA98NR, with and without metabolic activation (ECB IUCLID-PNA, 2000). A key study selected to fulfill this HPV Endpoint was conducted according to GLPs and conformed to OECD Test Guideline 471. It is considered “1-Reliable without restriction” and has been cited in Table 8 as well as extensively summarized in the Robust Study section of this Dossier.

Other *in vitro* mutagenicity assays conducted with PNA have provided mixed results. PNA was considered positive in the Japanese Rec assay and in a Mouse Lymphoma assay but negative in a CHO cell HGPRT forward gene mutation assay (ECB IUCLID – PNA, 2000); further, no genotoxic activity was reported when PNA was tested in an *in vitro* Unscheduled DNA Synthesis (UDS) assay or in an *in vivo/in vitro* DNA Synthesis test (ECB IUCLID – PNA, 2000). The absence of mutagenic activity was noted when PNA was tested in a secondary tier point mutation assay, the *Drosophila* germ cell test for sex-linked recessive lethal (SLRL) mutations (ECB IUCLID – PNA, 2000).

Conclusion: The Ames test HPV endpoint for PNA has been fulfilled. Further, the preponderance of the mutation data with mammalian cells and secondary tier assays indicate that PNA does not pose a mutagenic risk and no further testing is warranted.

Ames Test - o-Nitroaniline

A considerable number of Ames test assays have been conducted with ONA, several which fully meet international test method guidance (ECB IUCLID – ONA, 2000). Results have been negative (i.e. no mutagenic activity) in every *Salmonella* tester strain used, with and without metabolic activation. An Ames test conducted according to guideline OECD # 471 was selected to support this HPV Endpoint. It has been given a rating of “2-Reliable with restrictions” in that, while well documented, no information as to its compliance with GLPs was included in the literature citation from which it was taken.

Conclusion: The Ames Test Category Endpoint for ONA has been met and no further testing should be considered for the gene point mutation endpoint for this chemical.

Table 8. Genetic Toxicity of Category Members

Chemical	Ames Test- TA98, 100, 1535, 1537 +/- activation	Cytogenetics In Vitro	Cytogenetics In Vivo
o-Nitroaniline CAS NO. 88-74-4	Neg. w & w/o S-9.; all strains	Ambiguous- CHO Cells	Negative – mouse micronucleus assay (IP)
p-Nitroaniline CAS No. 100-01-6	Pos. TA98, w/o S-9. (border- line w S9)	Ambiguous - CHO Cells	Negative – mouse micronucleus assay (IP)

Chromosomal Aberrations - p-Nitroaniline

Several *in vivo* and *in vitro* studies have been conducted to assess the potential clastogenicity of PNA (ECB IUCLID – PNA, 2000). A Mouse Micronucleus test, fully complying with OECD Test Guideline 474 and considered “1-Reliable without restriction”, is presented in Table 8 as the key study to fulfill this HPV Endpoint requirement. A Robust Summary of this study can be found in section VII of this Dossier. No mutagenic response was seen in this secondary tier *in vivo* study.

Two *in vitro* CHO cell chromosomal aberration studies, including one which also evaluated Sister Chromatid Exchange potential of PNA, are also reported in the ECB IUCLID – PNA (2000). Weak, sometimes nonreproducible positive responses were observed at cytotoxic dosages while the potential influence of pH and ionic strength were not considered. Hence, these studies are considered to provide ambiguous results and of insufficient reliability for use in this assessment.

Conclusion: On the basis of a highly reliable Micronucleus study available with PNA, no additional testing is needed to fulfill this HPV Endpoint.

Chromosomal Aberrations - o-Nitroaniline

Two independently conducted Mouse Micronucleus tests, each administering ONA by the IP injection route, substantiated the absence of increased micronuclei formation at any test level (ECB IUCLID-ONA, 2000). While both of these studies meet study conduct and reporting sufficient to be considered fully reliable, we have cited (Table 8) and summarized (Robust Summary) one study as representative and thus the key study to fulfill this HPV Endpoint. This study fully complies with OECD Test Guideline 474, was conducted according to GLPs, and thus is considered “1-Reliable without

restriction”. The ECB IUCLID for ONA also cited an article reporting results of an *in vitro* chromosomal aberration study, as well as a mouse micronucleus assay using oral dosing. This report is considered unreliable as the paper itself questioned the legitimacy of the results. Thus, we have not included that report in this Dossier.

Conclusion: Based on availability of a fully reliable Mouse Micronucleus test, this HPV Endpoint for ONA has been fulfilled. No additional testing is warranted.

4.0 Reproductive and Developmental Toxicity

The Reproductive and developmental toxicity associated with the chemicals in this Category have been well studied. The sentinel chemical in this group, PNA, has undergone extensive testing for developmental toxicity in two species (rat and rabbit) and has been evaluated in a two-generation rat reproduction study (Nair et al, 1985, 1990). It has also been included in a preliminary developmental toxicity screen in mice (Hardin et al., 1987). Each of the PNA studies reported in Nair et al (1985) have been assessed as “1-Valid without restriction” as they fully met OECD testing and GLP guidance. The Two Generation rat Reproduction study is considered the key study to fulfill this HPV Endpoint for PNA, while the developmental toxicity studies are included as Supplemental information. Each of the adequately conducted studies has been summarized in Table 7.

Conclusion: Based on completion of the Two-Generation Rat Reproduction Study with PNA, no further testing is needed to meet this HPV Endpoint for this chemical and none is planned.

ONA has been evaluated in a comparative (to PNA) rat teratology study. This study has also been evaluated as being “1-Valid without restriction” and has been summarized in Table 7.

We believe sufficient data exist in this Category to provide an adequate evaluation for ONA based on similarity of mammalian toxicity between ONA and PNA and through use of corresponding reproductive toxicity data available on PNA. While no reproductive toxicity study has been conducted on ONA, a fully acceptable developmental toxicity study is available. Results of 4-week repeated dose studies by 2 exposure routes with ONA and PNA confirmed that the male and female reproductive organs are not target organs for either chemical. It is recognized that none of the ONA repeated dose studies meet the OECD acceptance criterion of 90 days test duration agreed upon to accommodate this endpoint. However, the following toxicological considerations justify the use of a “Read across” approach, using the PNA reproductive study in rats to substitute for similar unnecessary testing with ONA: (1) the comparative toxicity between ONA and PNA in similarly conducted acute and repeated dose mammalian toxicity studies (noting that ONA was always less toxic than PNA), (2) the lack of significant adverse findings in the ONA

developmental toxicity study, (3) the absence of reproductive effects associated with PNA exposure up to levels inducing other signs of toxicity, (4) extensive subchronic and chronic testing of PNA in multiple species, all of which failed to identify male or female gonads as a target tissue and (5) the highly controlled, closed system manufacturing and use environment associated with ONA already in place to minimize exposure potential and prevent methemoglobinemia.

Thus, we conclude that use of all available data in the Category approach, along with key studies with ONA itself, allows this HPV Endpoint to be completed without further unnecessary testing of ONA.

Table 9. Summary of Developmental Toxicity and Reproduction Studies with Category Members

Chemical	Study Type/Species	Dosage	Observations	Conclusion
o-Nitroaniline (ONA) CAS NO. 88-74-4	Rat Teratology – Gavage 25 /group	600 mg/kg	Maternal Toxicity: Body wt gain Food consump. Physical signs Terata-equivocal	NOEL for Embryotoxicity, Fetotoxicity, Terata (equivocal)
		300 mg/kg	Physical signs only	Absolute NOEL For Terata, embryotoxicity and fetotoxicity and NOEL for Maternal toxicity
		100 mg/kg	No findings	
p-Nitroaniline (PNA) CAS No. 100-01-6	Rat Teratology – Gavage 25/group	250 mg/kg	Maternal toxicity: Body wt. Gain Physical changes Spleen wt. Embryotoxicity: Resorptions Fetotoxicity: Fetal wts. Terata: External, soft tissue and skeletal	Teratogenic NOEL
		85 mg/kg	Maternal toxicity: Physical changes Spleen wt. Fetotoxicity: Fetal wts. No terata	

		25 mg/kg	No findings	Maternal toxicity NOEL Fetotoxicity NOEL
p-Nitroaniline PNA CAS No. 100-01-6	Rabbit Teratogenicity- Gavage 18/group	125 mg/kg	Maternal Toxicity: Deaths (7/18) Physical changes	NOEL for Terata, fetotoxicity, and embryotoxicity
		75 mg/kg	Maternal toxicity: Physical changes	NOAEL for Maternal Toxicity
		25 mg/kg	No findings	Unequivocal NOEL for Maternal Toxicity
p-Nitroaniline PNA CAS No. 100-01-6	Two-generation Rat Gavage Reproduction Study 15 males/30 females per group in F0 and F1 generations	9 mg/kg	F0/F1: all mating indices judged normal	NOEL for all reproductive endpoints
		2.5 mg/kg	No findings	
		0.25 mg/kg	No findings	

In summary, as seen previously in sections dealing with acute and repeated dose testing for mammalian toxicity endpoints, PNA has proven to produce the more significant comparative toxicity, hence the lower dosages used in the developmental toxicity studies listed (Nair et al, 1985). Albeit tested at lower dosages, only PNA exhibited significant developmental toxicity in the comparative rat studies. Severe maternal toxicity, along with embryotoxicity, fetotoxicity and frank malformations were observed at the highest dosage tested. Both maternal toxicity and fetotoxicity were observed at the mid dosage employed while the low dose selected was without treatment-related effect. As developmental effects were noted only at a dosage that produced significant maternal toxicity, PNA is not considered to cause a primary effect on fetal development.

PNA was found to be more toxic to rabbits than rats when tested in a rabbit developmental toxicity study (Nair et al, 1985). Frank maternal toxicity, including deaths, was observed at the highest dose tested, but there was no evidence of developmental toxicity observed, even at this test level.

ONA, on the other hand, produced substantive maternal toxicity in rats at the high dose tested, but produced no evidence of either embryotoxicity or fetotoxicity even at this level. Based on the study findings of a single malformation observed from two separate litters in the high dose group, it is unclear as to whether this was a treatment-related finding. The absence of production of this lesion in the previously discussed rat teratology study with PNA supports the conclusion that this was a spurious finding unrelated to ONA treatment.

PNA produced no evidence of adverse reproductive performance, including mating, fertility and pregnancy, littering or pup survival and development, in a Two-Generation rat Reproduction study using a top dosage which produced significant maternal toxicity (increased spleen weight, anemia, elevated blood methemoglobin levels) related to methemoglobinemia following chronic dosing (Nair et al, 1990).

Based on the results of these studies and the NOEL's derived, an adequate margin of safety exists at the recommended occupational exposure limits established for each of these Mononitroanilines.

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VII. ROBUST STUDY SUMMARIES

Appended

